

# A DESEEDED AVENA TEST METHOD FOR SMALL AMOUNTS OF AUXIN AND AUXIN PRECURSORS

By FOLKE SKOOG

(From the William G. Kerckhoff Laboratories of the Biological Sciences,  
California Institute of Technology, Pasadena)

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## INTRODUCTION

In 1927 Went isolated the growth promoting hormone, auxin, from the tip of the *Avena* coleoptile, and worked out the now well known *Avena* test method for its quantitative determination. By the use of this method the chemistry and many phases of the physiological action of auxin have been studied. In physiological work, however, the amounts of hormone involved are frequently so small that quantitative or even qualitative work has often been very difficult or impossible. In this article is presented a supplementary procedure with deseeded *Avena* seedlings whereby smaller concentrations of the hormone not detectable by the standard method can be quantitatively determined. By the use of this method it has also been possible to demonstrate directly the existence of substances capable of being converted into auxin by the plant. Some data relative to the presence of a precursor of auxin in *Avena* and synthetic precursors of hetero-auxin are included.

## *Description of Materials and Methods*

1. *The Standard Method.*—Since the deseeded method involves only changes in technique, it need not be described in detail, but rather in relation to the standard method which will first be reviewed. Oats of the pure line "*Sieges Hafer*" of Svalöv are dehusked, soaked in water for 2 hours, and laid out on wet filter paper in a moist dish in the dark room kept at 25°C., 90 per cent humidity, and free from any phototropically active light or toxic gases. The following day the seeds are planted in individual glass holders (Fig. 1) and placed with the roots in water, twelve plants in a tray. 2 days later when the coleoptiles are about 3 to 4 cm. long, they are ready to be used for tests. The plants are decapitated, about 0.5 cm. of the tips being removed, and the primary leaves are pulled loose from the

base. The plants are then allowed to stand for 40 minutes, so that the upper portions of the coleoptiles will become largely free from hormone, and so that any individual plants that might show curvatures due to handling can be detected and removed. At this time small agar blocks of standard size containing the hormone solution to be tested are applied to the cut surface on one side of the coleoptiles. After 110 minutes of application the plants are photographed as shadow pictures on bromide paper. The curvatures produced by the unilateral application of the hormone can then be measured with a goniometer from the pictures. For a single test the mean value of the curvatures of one row of twelve test plants is used. A complete description of the technique is given by Went (1928) and also in some detail (1935a).

It has been established by van der Weij (1932) and by Thimann and Bonner (1932) that under the conditions of the standard test the curvature is proportional to the concentration of hormone in the blocks of a given size in the range of concentrations from 1 to 15 or 25 degrees depending on the daily variation in the maximum angle. The amount of hormone diffusing from the block into the plant in the given 110 minutes of application, however, varies with the size and hormone concentration of the agar block in two respects: through the difference in contact surface and through the decrease in concentration gradient during the time of application. The point is illustrated in the original experiments by Went in which small agar blocks were used and from which 90 per cent of the hormone disappeared during the test, and those by Thimann and Bonner in which standard (eight times larger) blocks were used and from which only about 15 per cent disappeared in the same time. Thus in the former experiments the curvatures were indeed roughly a linear function of both the concentration and the amount of hormone applied, and therefore, also with these blocks smaller amounts of hormone could be determined. For other reasons, however, such as ease of manipulation and especially less susceptibility to change in volume by drying out, larger blocks are superior and have been adopted for standard tests. In this work in accordance with the specifications and units defined by Dolk and Thimann (1932) 1.5 per cent agar blocks of the dimensions  $\frac{8}{3} \times \frac{10.7}{4} \times 1.5$  mm. = 10.7 ml. have been used. The amount of hormone in one such block that will give a curvature of  $1^\circ$  under the above conditions corresponds to 0.4 A.E. (*Avena Einheiten*) and is therefore about  $1 \times 10^{-8}$  mg.

It should be noticed that under the above conditions only a fraction of the hormone applied has been utilized by the plant in the test, which must be completed within 2.5 hours after decapitation. After this time synthesis of auxin is resumed in the new physiological tip of the coleoptile, and since regeneration takes place especially on the side not in contact with the agar block (see page 330), the rate of bending of the coleoptile no longer remains proportional to the concentration of auxin applied. Hence changes in the procedure have been introduced. Most notable are those described by van der Weij (1931), who has developed special tools for decapitation and has introduced the use of a second decapitation

1 hour after the first one. This technique has also been used in the experiments below. It has been shown by Dolk (1926) that successive decapitations at 2 hour intervals prevent the regeneration of auxin in the plant. In accordance with this effect the double decapitation delays regeneration of auxin, and makes it possible to work with a larger number of plants. But the actual time of application of the blocks cannot be markedly increased by this method.

2. *The Deseeded Method.*—It has long been assumed, and will be shown here, that the auxin synthesized in the tip of the coleoptile is derived from a precursor transported from the seed. By deseeding, the

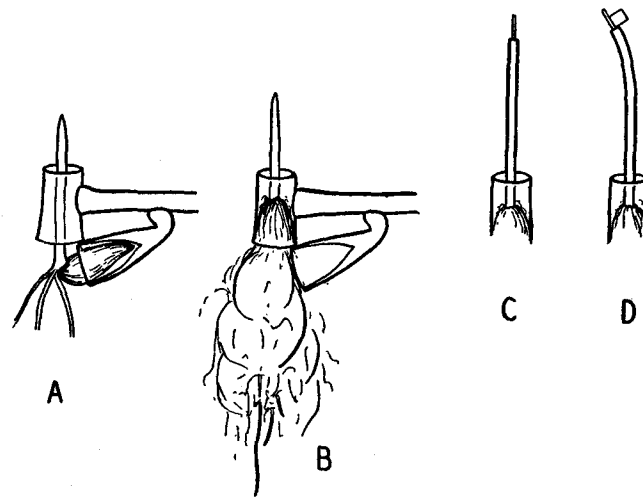


FIG. 1. Diagrams of steps in deseeded test showing plant *A*, ready to be deseeded; *B*, deseeded and replaced in holder; *C*, after second decapitation; *D*, after application of blocks and ready to be photographed.

source of auxin is removed; thus regeneration is prevented, and as a result more sensitive test plants are obtained. Hence, the changes in the standard procedure, briefly illustrated in Fig. 1, are as follows: on the 2nd day after germination, when the coleoptile is about 1.5 cm. long, the plant is taken from the holder, and the entire seed, with the exception of the lower half of the scutellum, is removed. A small piece of cotton is wound around the lower portion of the seedling, which is then reinserted in the holder with a pair of bent forceps (eye forceps). The cotton serves both to hold the plant and to insure a good water supply to the coleoptile. 12 to 18 hours later, as in the standard pro-

cedure, the plants are ready to be used for tests. Photographs may be taken singly or repeatedly at intervals at any time up to 5 or 7 hours after application of the blocks. A comparison of the curvatures obtained with this method and with the standard method is shown in Fig. 4.

#### *Physiological Factors Affected by Deseeding*

The effects of the removal of the seed on the subsequent development of the coleoptile are manifold. They will be discussed here mainly in relation to the growth response to auxin.

1. *Effect of Deseeding on Linear Growth.*—The effect of deseeding on linear growth has been studied by Went (1935*b*). He finds that the growth rate in deseeded plants is decreased about 40 per cent, and that this decrease is due to the lack of two factors, auxin and food. Furthermore, the application of high concentrations of auxin to intact deseeded plants causes about the same percentage increase in growth as in normal plants, but in deseeded plants the growth is more limited to the upper part of the coleoptile. In accordance with this behavior is the fact that in deseeded, decapitated coleoptiles, much sharper apical curvatures are obtained by the unilateral application of the hormone; *i.e.*, the basal parts do not grow and thus remain straight even after long times of application of the hormone.

The effect of deseeding on regenerative linear growth is shown in Fig. 2, in which is represented one of five similar experiments. Measurements of growth were made with a horizontal microscope on plants which had been marked with India ink into three zones. Each curve is the mean of three or four plants. The ordinates represent the sum of the total increase in length of two zones, which include almost the entire coleoptile in the decapitated plants and the corresponding two zones in the intact plants. Measurements were started 1 hour after decapitation. Curves I A and B represent intact and decapitated control plants respectively. Curves II and III A and B represent corresponding sets of plants which had been deseeded 1 and 10 hours respectively before the time of decapitation. From these curves the following facts are clear. By deseeding, growth is decreased both in intact and in decapitated plants. The amount of decrease is a function of the time after deseeding. In plants that were deseeded only 1

hour before decapitation, the regenerative growth in decapitated plants is only slightly reduced. In plants that were deseeded 10 hours before decapitation, regenerative growth is markedly inhibited. However, how far the decrease in regenerative growth depends on the lack of auxin alone or in addition on the lack of other factors, food, cannot be safely determined from the above curves.

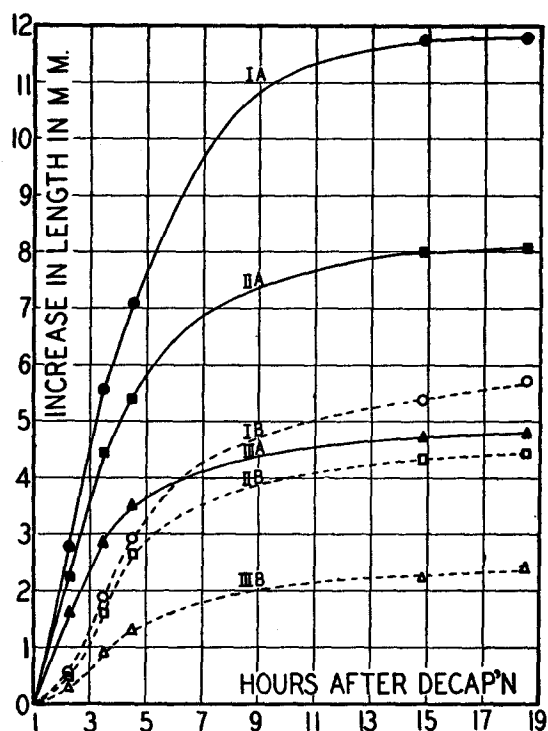


FIG. 2. Linear growth of normal and deseeded intact (solid line, A) and decapitated (broken line, B) plants. Curves I normal; curves II and III deseeded 1 and 10 hours before decapitation respectively. Measurements started 1 hour after decapitation.

2. *Effect of Deseeding on Geotropic and Auxin Curvatures.*—It will be shown that curvature growth, *i.e.* the difference in the relative growth of the two sides of the coleoptile, in deseeded and non-deseeded plants under the conditions described for the deseeded test, is independent of food and is a function only of the amount of auxin present

in the coleoptile. The following three types of experiments demonstrate this conclusion.

*a. Decrease in Auxin Synthesis after Deseeding.*—Beginning on the 2nd day after germination, plants were deseeded, thirty-six at a time, at successive intervals. Then at a given time, 3 mm. long coleoptile tips were cut off and placed on standard agar blocks, 15 tips per 12

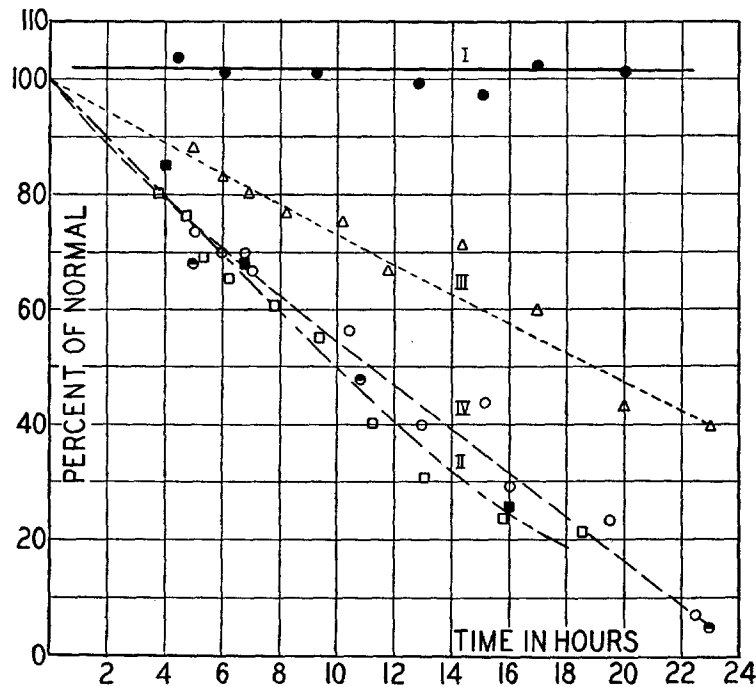


FIG. 3. Effect with time of deseeding on sensitivity to auxin, I; synthesis of auxin in tips, II; geotropic response in intact, III; in decapitated, IV, coleoptiles. All values are expressed as per cent of normal controls.

blocks, for 2 hours. The amount of hormone diffusing out from the different sets of tips was determined by the standard *Avena* method. All the blocks were tested at the same time with twenty-four test plants for each set of deseeded tips and forty-eight plants for the controls. The amount of hormone produced by deseeded plants expressed as per cent of that produced by normal plants is plotted against time after deseeding in curve II of Fig. 3, which represents the mean values

of several experiments. The curve shows that there is a continuous decrease in the rate of auxin synthesis after deseeding, and at least for a considerable period this decrease is closely a linear function of time.

*b. Sensitivity to Auxin.*—The relative sensitivity to auxin of deseeded plants at different times after deseeding, *i.e.* the capacity to produce curvatures in response to auxin applied unilaterally in blocks of moderate concentrations (5 to 20%) for 110 minutes as in the standard test, and compared with the curvatures produced by the application of the same concentrations of hormone in normal plants of the same group, is shown in curve I of Fig. 3. In this curve, which represents the mean values of many experiments with over 600 plants, the curvatures of deseeded plants, expressed as per cent of the curvatures obtained in corresponding control plants, are plotted against time after deseeding. It is clear that the sensitivity of deseeded plants is at least as great as that of normal plants. Only when the interval between deseeding and the test is made very long (not included in the graph) and also, which is probably more important, when the plants are deseeded in a very early stage of development, is there evidence of a distinct decrease in sensitivity. It appears from the curve that the sensitivity of deseeded plants may be slightly higher than that of normal plants. If this increase be real, it is probably due to the fact that in normal plants regeneration has begun less than 2.5 hours after the second decapitation (see also below). However, it should be pointed out that for moderate concentrations of hormone, this difference is so small that unless a very large number of tests are made, it is well within the experimental error.

*c. Decrease in Geotropic Curvatures in Deseeded Plants.*—It has been shown by Dolk (1926) that the geotropic curvature in normal intact and decapitated coleoptiles is controlled by the amount and relative distribution of auxin in the organ. Coleoptiles not producing auxin, as for example freshly decapitated plants, show no geotropic response. But similar coleoptiles to which auxin has been applied over the entire cut surface produce geotropic curvatures which are proportional to the concentration of hormone applied. Furthermore, the amount of hormone obtainable by diffusion from the upper and lower sides of geotropically stimulated coleoptiles is proportional respectively to the growth of the two sides. Since it was shown in section 2 *b* that the

sensitivity to auxin is not decreased by deseeding, it can be said with fair certainty that the relations between auxin and geotropic curvature, established by Dolk for normal plants, hold also in deseeded plants. Thus in conjunction with the experiments of sections *a* and *b*, a large number of determinations were made of the relative geotropic response in deseeded plants. At definite times after deseeding, de-

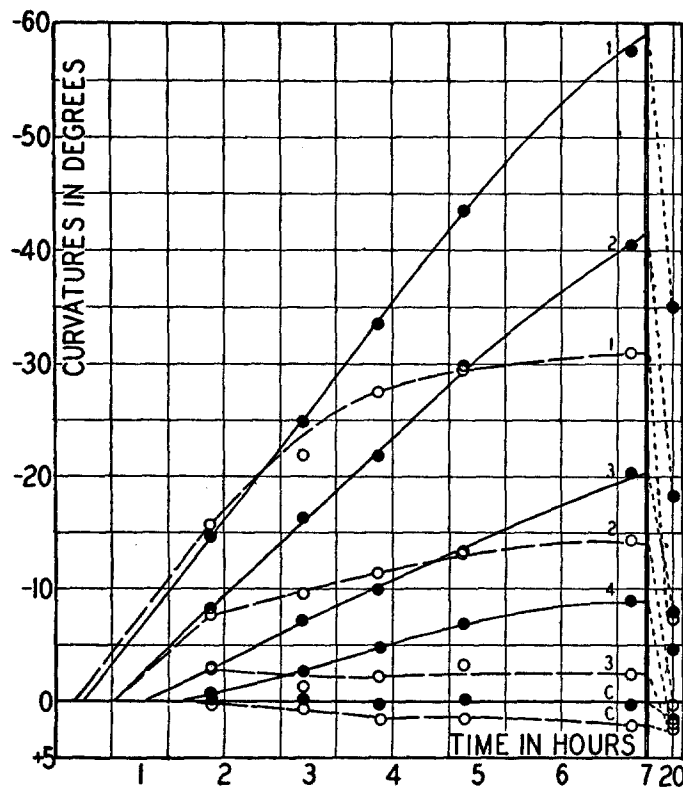


FIG. 4 A

seeded and normal plants of the same group were placed horizontally. The geotropic curvatures produced in a given time, 1 hour for intact plants and 4 hours immediately after decapitation for decapitated plants, were measured from photographs taken at the end of the specified times. The curvatures of deseeded plants expressed as per cent of those of normal plants and plotted against time after deseeding are



shown in curves III and IV of Fig. 3, representing intact and decapitated plants respectively. The curves demonstrate that in both intact and decapitated coleoptiles of deseeded plants there is a decrease in geotropic response proportional to the decrease in auxin synthesis. Thus, if the plants are decapitated about 15 hours after deseeding, subsequent regeneration of auxin is nearly completely prevented.

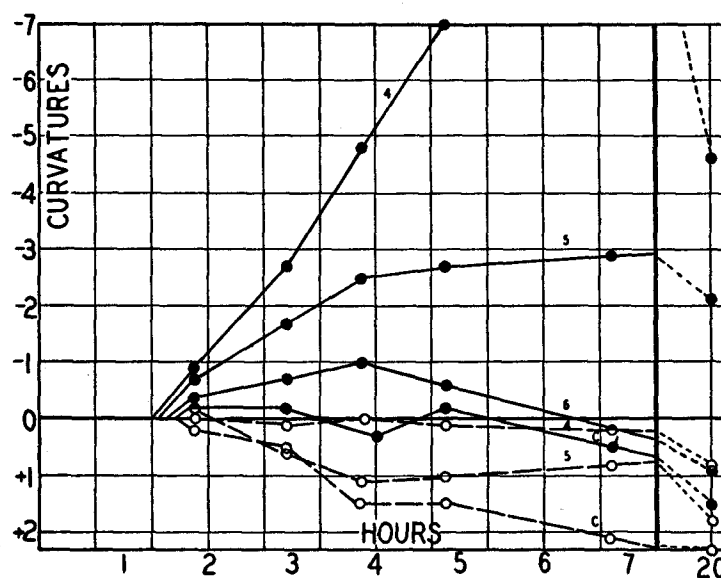


FIG. 4 B

FIGS. 4, A and B. Comparison of deseeded (solid lines) and standard (broken lines) tests for different concentrations of hormone.

Curves 1 2 3 4 5 6 and C  
correspond to concentrations of 1 1/2 1/6 1/18 1/54 1/108 and 0  
respectively.

From a consideration of the data in Fig. 3 as a whole it is possible to draw one further conclusion. Since the decrease in geotropic curvature in deseeded plants is independent of the sensitivity of the coleoptiles and thus depends only on the decrease in auxin synthesis, and since the relative decrease in auxin synthesis in regenerating decapitated coleoptiles is very nearly the same as that in decapitated tips and proportional to that in intact tips, it follows that the mechanism

of auxin synthesis in the tip of the intact coleoptile and the mechanism of regeneration of auxin in the new physiological tip of the decapitated coleoptile are identical.

3. *Comparison of the Deseeded and the Standard Tests.*—We are now in a position to consider the relations between the deseeded and the standard tests in terms of physiological factors affected by deseeding. The curves in Fig. 4 *A* and *B*, obtained in one of six experiments with practically identical results, represent curvatures in degrees plotted against time in hours of unilateral application of hormone of different concentrations to deseeded and to normal plants. For the sake of clearness the lower concentrations are plotted separately on a larger scale in *B*. In this experiment the deseeding was done 15 hours before the time of the first decapitation. Photographs were taken after 110 minutes of application as in the standard test and then at successive intervals.

*a. Regeneration and Temperature Effects.*—By comparing each continuous line (deseeded plants) with its corresponding broken line (normal plants), it becomes clear that for moderate concentrations of hormone above  $3^{\circ}$ , the curvatures are the same in both tests for the first 2 hours. However, after this time regeneration sets in. As a result, in normal plants the rate of bending is decreased, so that the curvatures recede, remain constant, or continue to increase at a slower rate, according to the concentration of hormone applied. In deseeded plants, where regeneration is practically completely lacking, the curvatures continue to increase linearly with time for several hours, or if the concentration of hormone is smaller, until the supply of auxin from the blocks has been largely depleted. As a matter of fact, in normal plants a small amount of regeneration takes place earlier than 2.5 hours after the second decapitation. Thus in the standard test, when blocks of low concentrations are applied, practically no curvatures will appear; when blocks of very low concentrations or pure agar blocks are applied, positive curvatures, *i.e.* in the direction towards the blocks, will occur. The cause of these positive curvatures will be clear from a consideration of the precursor of auxin, which diffuses out into the agar blocks and is not immediately converted into auxin. Also in deseeded plants small positive curvatures may be obtained by the unilateral application of pure agar blocks, but the effect is much

less than in non-deseeded plants. Thus for determinations of small concentrations of hormone, deseeded plants are relatively even more sensitive than for higher concentrations. This additional sensitivity appearing already within the first 2 hours of application is clearly brought out in Figs. 5 and 6, in which the curvatures obtained in two experiments as determined by deseeded and standard tests are plotted

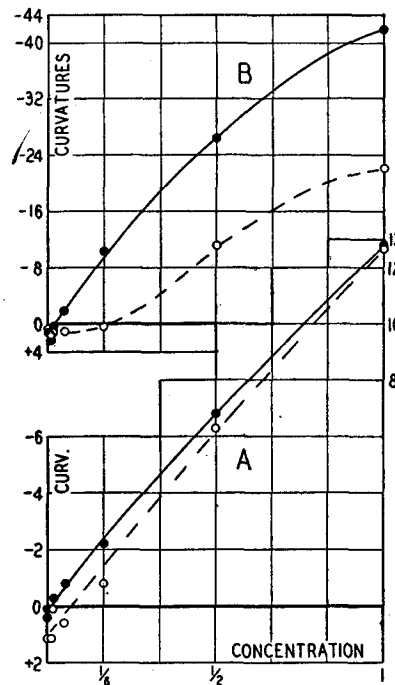


FIG. 5. Comparison of curvatures obtained by deseeded (solid line) and standard (broken line) tests plotted against concentration of hormone applied. A, photos taken after 110 minutes; B, after 390 minutes application.

against concentration of hormone applied. The curves obtained by the use of normal plants intersect the abscissa some distance away from the origin, whereas the curves obtained with deseeded plants come very close to the origin. The distance from the origin to the point of intersection of the curve with the abscissa for normal plants increases very rapidly with increase in temperature as does regeneration. But the question whether regeneration is the entire cause of the

decreased sensitivity or whether there is in addition a small destruction of auxin by the plant (see Van Overbeek (1936)) must be left open, because the curvatures involved are so small that the two effects cannot be clearly differentiated in these experiments. At higher temperatures ( $27^{\circ}$ ) autotropism, perhaps due to regeneration, becomes

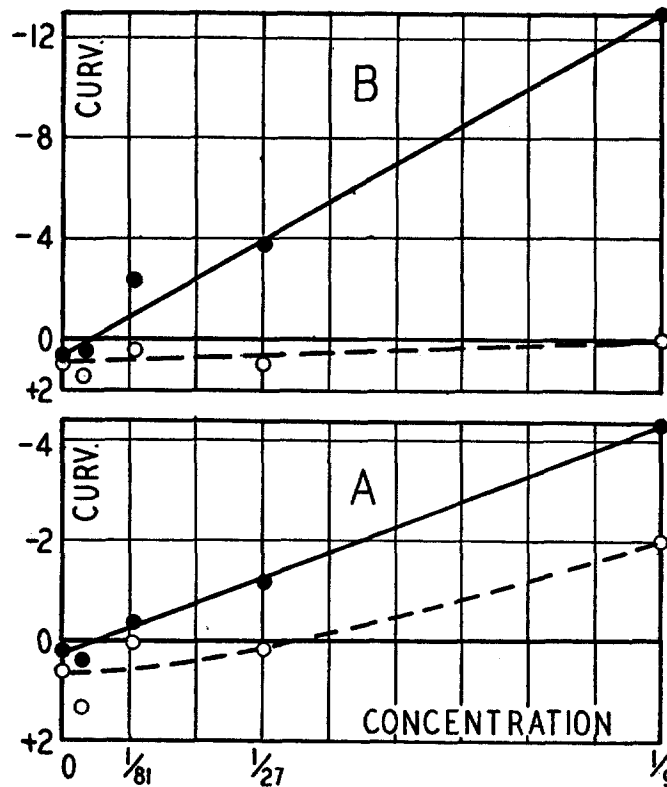


FIG. 6. Experiment similar to Fig. 5 showing lower concentrations on a larger scale. A, photos taken after 110 minutes; B, photos taken after 300 minutes of application.

noticeable also in deseeded plants, so that after about 4 hours the linear relationship between curvatures and time of application becomes less pronounced.

*b. Less Physiological Aging.*—Also contributing to the higher sensitivity of deseeded plants, especially long times after decapitation, is

the decrease in physiological aging. Du Buy (1933) and Went (1935b) have shown that decapitated coleoptiles prevented from synthesizing auxin and not supplied with auxin for several hours, gradually become less sensitive to subsequently applied auxin. This effect, "physiological aging," is due to the increase in thickness and loss of plasticity of the cell walls. By deseeding, the materials for secondary cell wall formation are largely removed, and the walls of the coleoptiles remain thin and plastic even though the actual amount of auxin in them is less than in normal plants.

*c. Limits for Concentrations and Amounts of Hormone.*—In general it can be said that with the deseeded method about ten times as small concentrations can be determined as can be detected with the standard test. This fact is readily understood, if we consider that in the standard test only 15 per cent of the auxin passes from the agar block into the plant, whereas in the deseeded test, if photographs are taken after about 5 hours, nearly all the auxin in the block is utilized. For example, if two blocks containing very low concentrations are placed one on top of the other on a deseeded test plant about twice as large curvature is obtained as by a single block. In the standard test on the other hand, van der Weij found the curvature to be independent of the size of the block. However, it is clear that in the deseeded as in the standard test there is a distinct limit to the concentration of auxin that can be detected. If the limit for the standard method is taken as  $1 \times 10^{-8}$  mg. per block, then the limit for the deseeded method is about  $1 \times 10^{-9}$  mg. per block.

*d. Effect of Light.*—Since white light has a marked effect on the growth of the *Avena* coleoptile, its influence on curvatures when successive photographs are taken must also be considered. These experiments, which have a bearing on the light growth reaction, have been done by Van Overbeek, and the data given in Fig. 7 have been kindly contributed by him. In the standard test the plants are not exposed until the end of the experiment. In the deseeded test, however, it is often desirable to make a series of estimations of the curvatures of a given set of plants. Van Overbeek (1936) has shown that the amounts of light necessary for taking photographs partially inhibit regeneration. In accordance with this as shown by curves A, Fig. 7, the increase in curvature with time obtained from a series of photo-

graphs at consecutive intervals of a given set of normal plants is greater than that obtained from plants of the same group, but of which a different set of plants, not previously exposed to light, is used at each corresponding interval. Curves B of the figure represent the identical

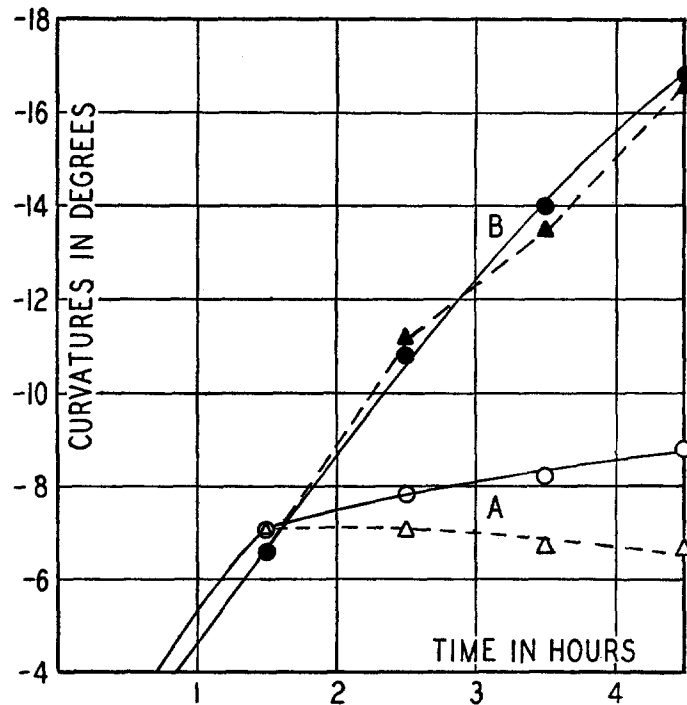


FIG. 7. Effect of light on curvatures in normal (curves A, open points) and deseeded (curves B, solid points) plants. Values represented by circles (solid lines) are from repeatedly exposed plants. Values represented by triangles (broken line) are from different sets of plants of the same group and not previously exposed.

experiment with similar plants deseeded 20 hours before the auxin was applied. From the close agreement between the curvatures of successively exposed and not exposed plants, it is clear that the amount of white light necessary for photographing has no effect on the rate of bending of deseeded plants.

*Application of the Deseeded Test for Auxin Determinations*

As illustration of the deseeded test, two examples will be given which include some data previously not easily obtainable.

1. *Determination of Auxin in Primary Leaves of Avena*.—Primary leaves of 4 day old plants grown in the dark room were pulled out of the coleoptiles and placed with their bases on agar blocks, twelve leaves per twelve blocks, for 2.5 or 4.0 hours. The blocks were then tested on deseeded plants. Control agar blocks on which no leaves had been placed were tested at the same time. For comparison one standard test was also made. The results of three experiments with

TABLE I  
*Auxin from Primary Leaves*

Exp. No.	Time of		Mean curvatures from		
	Diffusion into block	Application in deseeded test	Blocks with diffusate in test		Plain agar in deseeded test
			Deseeded	Standard	
	<i>hrs.</i>	<i>hrs.</i>			
1	2.5	8.0	-3.9	0.0	+0.1
2	4.0	6.0	-4.5	—	+0.4
3*	3.5	5.0	-2.3		
			-2.1		
			-2.6	+0.7	+0.7
			-2.8	+0.7	

\* In Exp. 3, 18 leaves per 12 blocks were used, but values are divided by 1.5 to give degrees in terms of 12 leaves per 12 blocks.

leaves grown under different conditions, and which are therefore not comparable with each other, are summarized in Table I. With the deseeded test the presence of auxin is clearly demonstrated, whereas with the standard test none can be found, and, in fact, has been thought to be absent. Furthermore, the close agreement between the values obtained in different tests of the same experiment shows that the method gives quantitative results.

2. *Determination of Auxin in Coleoptile Sections*.—It has been shown by Thimann (1934) by the use of chloroform extractions from a large number of plants that in coleoptile sections auxin is present in small amounts. This finding has been confirmed by placing 0.3 cm. long

coleoptile cylinders on agar blocks, which were subsequently tested with deseeded plants. As shown in Table II, the amounts of auxin obtained are large enough to give quantitative measurements. Comparable experiments with the standard test give at best only a perceptible curvature.

For a computation of the actual amounts of hormone obtained in the above experiments, it is best to compare the curvatures directly with those obtained in similar tests with successive dilutions of an

TABLE II  
*Auxin from Coleoptile Sections.*  
*3 Mm. Long Sections Placed 20 Sections Per 12 Blocks*

Exp. No.	Time of diffusion into block	Mean curvatures from			Time after application of blocks to 2nd photo
		Diffusate blocks		Plain agar 2nd photo	
		1st photo	2nd photo		
	<i>hrs.</i>				<i>hrs.</i>
1 <i>a</i>	4.0	-2.3	-5.9		
<i>b</i>		-2.7	-6.2	+1.2	5
2	3.2	-1.2	-4.8	+1.4	8
3	3.5	-0.4	-3.4	+1.8	7
4 <i>a</i>	4.0	-1.6	-5.7		
<i>b</i>		-1.3	-3.5	+1.6	10
5 <i>a</i>	4.0	-1.0	-4.1		
<i>b</i>		-0.7	-2.7	+2.2	5
6 <i>a</i>	4.0	-0.5	-3.0		
<i>b</i>		-0.5	-3.4	+2.6	9

auxin solution of known, relatively low concentration. From such a comparison it was estimated that the auxin obtained from the primary leaf is of the order of 0.05 A.E. per leaf per hour, and that from sections about 0.03 A.E. per section per hour. These amounts are only 5 or less per cent of the amount obtainable from the coleoptile tip per hour.

The above experiments, although not carried out in detail, indicate the possibilities of the application of the deseeded test to work concerned with the presence and relative distribution of the growth hormone in plant tissues. They also bring out the fact that the high sensitivity of the deseeded test holds for auxins in general and is not



limited to synthetic hetero-auxin, which was used above exclusively in the development of the method.

### *Precursors of Auxin*

Another use of the deseeded method and for which purpose it was originally designed is the detection of precursors of auxin.

1. *Demonstration of a Precursor of Auxin in the Coleoptile.*—It has been shown above that there is a limit to the concentrations of hormone that can be detected by the deseeded test. If the concentrations of hormone are so small that they will not cause distinct curvatures to appear within the first 5 to 6 hours after application, curvatures will not appear at any time later. Furthermore, it is well known that the transport of auxin in the *Avena* coleoptile is strictly polar in the direction from the tip towards the base. Even with the deseeded test, no detectable concentration of hormone has been found to be actively transported in the opposite direction. In accordance with this fact when coleoptiles are decapitated, the primary leaves removed, and agar blocks are placed over the entire cut surface of the stumps for 2 or more hours, and these blocks are then tested on deseeded plants, as expected, no curvatures are obtained within the first 5 or 6 hours of application. However, some time later, 10 to 20 hours after application, distinct negative curvatures are obtained. Results of some determinations are given in Table III. In the determinations made so far, the curvatures have varied considerably from one experiment to the next, but frequently the mean curvatures have been between 4 and 8°. The variability in the magnitude is to be expected, since the optimal experimental conditions must be governed by several factors, whose nature is as yet unknown.

Perhaps a more striking way of demonstrating the precursor, which brings out the difference between it and auxin itself, is by the following arrangement. Sets of twenty coleoptile sections of given lengths, 5 or 3 cm., are placed with the bases either down or up, but all of a given set in the same direction, between two 12 times standard size agar blocks. After a given time, varying between 2 and 4 hours, the agar blocks are removed, cut each into twelve standard blocks, and tested on deseeded plants. The curvatures produced are measured

from photographs taken at intervals. Results will not be given in detail, but the mean values of some fifteen separate determinations are presented by curves I, II, and III of Fig. 8. These curves represent the curvatures plotted against time of application obtained from blocks previously applied to the basal (I) and apical (II) ends of sections and from control agar blocks (III) respectively. A comparison of the curves shows that the material diffusing out at the apical surface is different from that diffusing out at the basal surface in that it will only cause curvatures a long time after application. From the data

TABLE III  
*Precursor of Auxin from Coleoptiles*

Exp. No.	Time of		Mean curvatures from	
	Diffusion into blocks	Application in test	Apically applied blocks	Plain agar blocks
1	5.0	hrs.		
		17	-2.6	
		20	-6.0	
		20	-4.0	
2	3 to 4	20	-7.0	+0.4
		24	-6.0	
			-7.2	
3	1.5 or 3.5	16	-4.6	+0.3
			-6.5	
			-4.9	
			-4.0	
4	?	?	-5.9	+0.2
			-1.6	
			-2.3	+0.8

in Table III and curve II of Fig. 8 the conclusion is drawn that the growth promoting substance from the "apical" blocks is a precursor of auxin capable of being transported in the apical direction in the plant and capable of gradually undergoing a chemical change into auxin, whereas auxin itself can be transported in the plant only in a basipetal direction.

*The Relation between Positive Curvatures and the Precursor.*—It was shown above that by unilateral application of plain agar blocks to decapitated plants small positive curvatures are produced. As early

as 1927 Gorter pointed out that these curvatures are not due to growth inhibiting substances, but are correlated with the regeneration of auxin in the new physiological tip. Why and by what mechanism regeneration is affected has not been made clear. From a determination of the amount of regeneration in coleoptiles with and without agar blocks, and from a consideration of the precursor of auxin, these questions will be answered. About 150 plants were decapitated. To half this

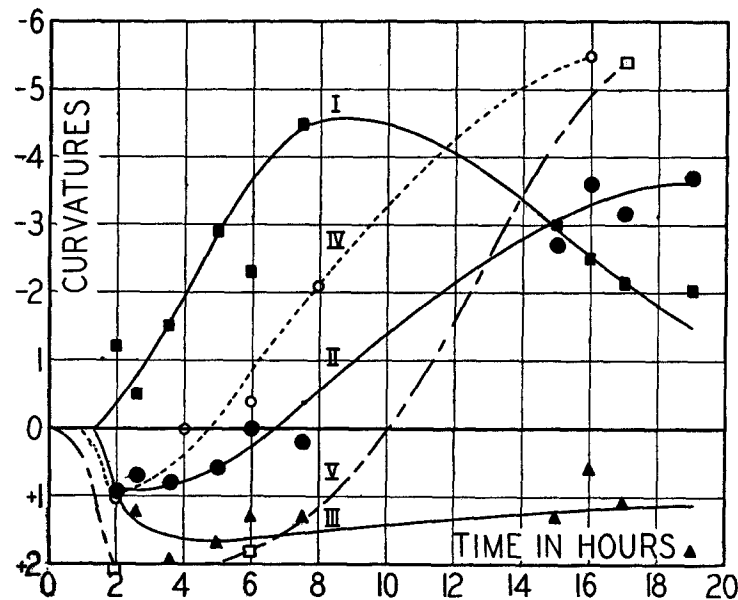


FIG. 8. Illustration of curvatures obtained in deseeded plants by the application of blocks containing auxin from coleoptile sections (curve I); precursor of auxin from coleoptile sections (curve II); pure agar (curve III); tryptophane (curve IV); and indolethyl amine (curve V).

number plain agar blocks were applied to the entire cut surface of the coleoptiles immediately after decapitation; the other half was used for controls. Between 2 and 3 hours later 1.5 to 2.0 mm. long apical sections were cut off and placed on agar blocks, twenty-four sections per twelve blocks, for 2 hours. The amount of auxin produced by the sections was determined by testing these blocks on deseeded plants. The results (Table IV) show that in the apices of plants on which agar

blocks had been placed, the production of auxin was significantly less than in the controls without agar blocks. Furthermore, tests of the agar blocks which had been placed on the apical surfaces showed no trace of auxin, but on the other hand indicated the presence of the precursor. The mechanism of the formation of positive, differential regeneration, curvatures is therefore as follows. On the side of the coleoptile in contact with the agar block a considerable fraction of the precursor of auxin diffuses out into the agar block and will not be immediately converted into auxin. On the opposite side of the coleoptile precursor accumulates and is converted into auxin. The relatively larger auxin production on this side makes possible a corresponding increase in growth, which causes a positive curvature.

TABLE IV

*Regeneration in Decapitated Coleoptiles with and without Agar Blocks Applied to the Cut Surface*

Time of application of blocks	Mean curvatures with mean error from sections previously		Difference in degrees
	With blocks	Without blocks	
<i>hrs.</i>			
3.0	$-4.1 \pm 1.2$	$-9.4 \pm 1.2$	$-5.3 \pm 1.7$
2.5	$-2.8 \pm 0.8$	$-9.8 \pm 1.2$	$-7.0 \pm 1.4$
2+	$-4.9 \pm 0.6$	$-7.1 \pm 1.2$	$-2.2 \pm 1.3$
Curvatures from plain agar blocks $+1.4 \pm 0.3$			

2. *Precursors of Hetero-Auxin.*—Since the chemistry of the auxin occurring in the *Avena* seedling is exceedingly complicated, because of the complex structure and instability of the active substance, no data on the chemical nature of the precursor have been obtained. However, independent evidence, the chemical nature of which is better understood, will now be presented in support of the conclusions drawn from the experiments in section 1.

It has been shown by Thimann that chemically pure tryptophane will not produce curvatures when tested by the standard *Avena* method. But when tryptophane is applied to sections in solution, it will promote elongation. Furthermore, from experiments on the synthesis of hetero-auxin by *Rhizopus suinus* Thimann (1935a) has

shown that tryptophane is a precursor of hetero-auxin. The mechanism of the transformation is an oxidative deamination and decarboxylation.

What happens when very dilute tryptophane blocks are applied unilaterally to deseeded plants? It is evident from curve IV of Fig. 8 that tryptophane behaves in exactly the same manner as the precursor from the plant, curve II, with the exception that the effect of tryptophane is greater. By choosing the proper concentration of tryptophane, the same type of curve can also be obtained with normal test plants, *i.e.* the amounts of hetero-auxin formed from tryptophane are large enough to more than balance the effects of regeneration and autotropism, and may, in fact, cause the plants to be in a state of active bending for more than 36 hours. It has been shown by Kögl and Kostermans (1935) that indolpyruvic acid has auxin activity. Thus the possibility exists that this acid rather than hetero-auxin ( $\beta$ -indolacetic acid) is formed from tryptophane. However, these authors point out that the apparent activity of indolpyruvic acid might likely be due to its breakdown into indolacetic acid. They calculate that 1 per cent breakdown will account for the measured activity.

Another substance, indolethyl amine, kindly synthesized by Dr. J. Koepfli, has been found to be very suitable for precursor experiments. This compound is completely lacking in growth promoting activity, but in contact with the cut surface of the plant it can become activated and will then produce curvatures; see Fig. 8, curve V. It is superior to tryptophane in that it is more slowly activated, does not contain a carboxyl group which is possessed by all known active substances, and its only active degradation product is hetero-auxin. All lower degradation products are known to be inactive. It has further been found that by placing agar blocks containing either tryptophane or  $\beta$ -indolethyl amine in contact with the apical surfaces of a large number of coleoptile cylinders for a short time they become activated, so that when the blocks are subsequently applied to test plants, they will cause large auxin curvatures, which start to appear almost immediately after application.

The experiments described in this section, although dealing with substances evidently not identical with the precursor of auxin obtained

from *Avena*, nevertheless lend strong support for the evidence given above for its existence and behaviors. They show that the delayed curvatures obtained above are due to an activation through chemical changes in the substances applied, and are not merely the result of any possible differences in the rates of transport in the plant of these substances and the auxins. The activation of the precursor takes place most likely in the agar block in contact with the plant or extracellularly at the cut surface, since if the activation took place exclusively intercellularly, active substance would not be recoverable in the apical blocks. Other experiments, not described here, exclude the possibility of activation through bacterial action. Considerable evidence indicates that in the case of the synthetic precursors of hetero-auxin the nature of the activation process is fairly certainly an oxidative deamination. How far the relationship existing between these synthetic precursors and hetero-auxin can be extended to explain the relationship between the precursor and auxin in the plant is as yet a speculative matter. A more complete study of the chemical nature, specific physiological activities of the precursor of auxin, and the mechanism of its transport and activation in the plant is in progress.

It has been pointed out by Thimann (1935*b*) and by Haagen-Smit and Went (1935) that a clear distinction must be made between true auxins and such substances as may promote growth but are not capable of being polarly transported in the plant. It has further been shown by Michener (1935) that auxins must be distinguished from such substances as may indirectly affect the physiological activity of auxin in the plant. In addition to these two groups must now also be considered a third group of substances capable of becoming activated by the plant into true auxins.

#### SUMMARY

The main results presented in this article may be summarized as follows:

1. A test method with deseeded *Avena* seedlings for small concentrations of auxin and precursors of auxin has been described.
2. This method makes possible quantitative determinations of about ten times as low concentrations of hormone as can be obtained with the standard method. (a) Through an increase in the time of the

test, so that nearly all the hormone applied can be utilized. (b) Through an increase in sensitivity of deseeded plants to unilaterally applied small concentrations of hormone.

3. The effect of deseeding in relation to curvature growth is primarily the prevention of auxin regeneration through the removal of the material for auxin synthesis, and in addition the prevention of physiological aging.

4. The mechanism of auxin synthesis in the tip of the coleoptile and the mechanism of auxin regeneration in the new physiological tip have been shown to be identical.

5. The application of the deseeded method is illustrated by determinations of auxin in primary leaves and coleoptile sections of *Avena* seedlings.

6. The deseeded method has been used as a test method for precursors of auxin obtainable from the coleoptile and from other sources. The method further makes possible a distinction between auxins and these substances which may become activated by the plant.

7. Evidence for the existence of a precursor of auxin in the plant is given (a) indirectly by determinations of the decrease in auxin synthesis in deseeded plants. (b) Directly by its isolation from the plant.

8. Precursors of hetero-auxin are demonstrated; their chemical nature and activation are briefly considered.

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